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Association of Ecogeographical Variables and RAPD Marker Variation in Wild Potato Populations of the USA

A. H. del Rio, J. B. Bamberg,* Z. Huaman, A. Salas, and S. E. Vega

ABSTRACT

The goal of germplasm conservation in genebanks is to maximize genetic variation. Collecting explorations would be more efficient if factors that predict areas and habitats associated with greater genetic differences and diversity could be identified. Therefore, the objective of this research was to investigate whether ecogeographical variables have significant associations with patterns of genetic variation in wild potato populations. This study examined 96 wild potato populations collected from the southwestern USA. These were 43 populations of *Solanum fendleri* ($2n = 4x = 48$) and 53 populations of *S. jamesii* ($2n = 2x = 24$). These species represent two of the most predominant breeding systems found among *Solanum* species: tetraploid inbreeders and diploid outcrossers, respectively. Random amplified polymorphic DNA (RAPD) markers were used to assess populations in two ways: determination of simple genetic difference between pairs of populations, and genetic diversity of a population based on the frequency of that population's RAPD markers in the whole set. Results from 2282 comparisons indicated that patterns of genetic differences were not associated with any differences in ecogeographical structure assessed. Remarkably, even geographical separation of populations, a parameter usually considered important when collecting germplasm, did not predict genetic differences very well. Latitude, longitude, and heat-related factors significantly predicted genetic diversity in *S. fendleri* but not in *S. jamesii*. This experiment revealed few associations between ecogeographic parameters and genetic variation in the wild. It follows, therefore, that one should collect many populations and incorporate a manageable subset into the genebank on the basis of empirical measurements of genetic diversity.

A.H. del Rio and S.E. Vega, Dep. of Horticulture, Univ. of Wisconsin-Madison, 1575 Linden Drive, Madison WI 53706; J.B. Bamberg, USDA-ARS, Vegetable Crops Research Unit, Inter-Regional Potato Introduction Station, 4312 Hwy. 42, Sturgeon Bay, WI 54235; Z. Huaman and A. Salas, International Potato Center, P.O. Box 1558, Lima 100, Peru. Reported use of brand name products does not imply an endorsement by USDA. Received 1 May 2000. *Corresponding author (nr6jb@ars-grin.gov).

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THE MAIN GOAL OF CROP GERMPLASM COLLECTIONS is to preserve potentially useful sources of genetic variation for future use (Cohen et al., 1991). Thus, research that addresses strategies to maximize the conservation of genetic diversity is fundamental. Altieri and Merrick (1987) and Brown et al. (1997) recognized that there are problems associated with inadequate sampling procedures during explorations and lack of representation of the total gene pool. However, the genebanks' resources to address these problems are usually limited.

Ferguson et al. (1998) recognized that sampling would be more efficient if collecting trips had clearly defined target areas and habitats. An approach to identify these areas is to undertake ecogeographic studies preceding explorations, since plant populations may be expected to exhibit structured genetic variation across their geographical range (Antonovics, 1971; Loveless and Hamrick, 1984). At the present time, the common and historic practice in explorations is to sample as many different environments as possible (Brown, 1978; Marshall and Brown, 1975).

Zoro et al. (1998) pointed out that although sampling methods have been designed using probability methods combined with population genetics theory (Crossa et al., 1993), these results can provide only rough predictions when basic information about a species reproductive biology is unstudied. Hamrick et al. (1991) stressed that there is considerable variation among plant species for ecological traits that influence the distribution of genetic variation, so a genetically effective management strategy for one species may not be effective for another. Therefore, detailed (and real) studies on ecology, popu-

Abbreviations: DI, diversity estimate; GD, genetic distance; NRSP-6, Inter-Regional Potato Introduction Station.

lation biology, genetics, and reproductive biology are essential to plan adequate sampling strategies (Hawkes, 1971; Yonezawa and Ichihashi, 1989).

Germplasm conservation is extremely important in the case of potato species. The cultivated potato, *Solanum tuberosum* L., is the most important tuber crop in the world and is one of the four most valuable crops worldwide. Germplasm with adaptation to different climatic and cultural conditions has been essential to the development of improved varieties (Ross, 1986). The U.S. Potato Genebank (NRSP-6) preserves nearly 5000 different accessions of potato and its wild relatives (Spooner and Bamberg, 1994). *Solanum* species have a wide range of ecological and geographic distributions in the Americas. Therefore, guidelines for relating environmental variables to genetic diversity would significantly enhance collection efficiency.

In recent years, molecular markers such as RAPD markers have been confirmed as efficient tools for estimating genetic variation among genotypes of any organism (Williams et al., 1990). These markers have the potential to measure variation with good coverage of the entire genome. RAPD markers have clearly resolved patterns of diversity of many diverse types of plant populations and germplasm collections (del Rio et al., 1997a,b; Link et al., 1995; Virk et al., 1995; Hormaza et al., 1994).

The main objective of the present study was to correlate genetic variation observed in natural wild potato populations in the USA with a series of different ecological, geographical, and reproductive variables. Since these species present two of the most important breeding systems observed in *Solanum* species, information from these comparisons may provide general insights for future germplasm explorations for other species.

MATERIALS AND METHODS

Plant Materials

Ninety-six wild potato populations, collected from 1992 to 1997, from different geographical regions of the southwestern USA, were examined. (Bamberg et al., 1996, 1997). To maintain genetic integrity, these populations were clonally maintained at the Inter-Regional Potato Introduction Station (NRSP-6) at Sturgeon Bay, WI. Plant Introduction (PI) numbers, collector codes, and collection-site coordinates are presented in Table 1. *Solanum jamesii*, a diploid ($2n = 2x = 24$) outcrossing species was represented by 53 populations; and *S. fendleri*, a tetraploid ($2n = 4x = 48$), self-pollinating species was represented by 43 populations (Bamberg and Martin, 1993). More detailed information on these populations such as habitat, collection dates, and special observations is available through the USDA National Plant Germplasm System and Germplasm Resources Information Network database.

DNA Isolation

DNA was isolated from bulked fresh leaf tissue from each population according to a procedure modified from that described by Williams et al. (1994) in which potassium ethyl xanthogenate served to liberate DNA. Extracted DNA was dissolved and stored in TE 1× buffer (Promega, Madison, WI) at −20°C, and quantified by fluorometry using a TKO 100 Mini-Fluorometer (Hoefer Scientific Supplies, San Francisco, CA). Bulked DNA samples were produced from all individuals collected for each population (see Table 1).

RAPD Markers and PCR Amplification

Primers representing 10 random nucleotide sequences, obtained from Operon Technologies (Alameda, CA), were used in the RAPD assay. PCR amplifications were performed in 15-μL reaction volumes as described in del Rio et al. (1997a). All RAPD products were fractionated by electrophoresis in 1.5% (w/v) agarose gels and visualized by ethidium bromide staining 0.5 μg/mL in 1× TAE buffer.

Data Analysis

For each population, polymorphic RAPD markers were scored as present (1) or absent (0). Genetic variation of potato populations was measured as genetic distance (GD) between paired populations. GD coefficients were calculated as the complement of the simple matching coefficient: the number of loci with matching band status divided by the total number of loci assessed (Sneath and Sokal, 1973).

Genetic variation also was measured by means of a genetic diversity index (DI). This was designed to identify populations rich in rare genetic markers. Within each species, the frequency of each RAPD marker over all populations was determined. Each population was assigned a score for that marker equal to the frequency of the marker's presence or absence, according to whether it was present or absent in that population. Thus, a population having a marker which was present in 5% of the populations studied would be assigned a score for that marker of 0.05, while a population not having that marker would be assigned a score of 0.95.

The DI was calculated in two ways: The standard deviation of the marker score (SD_f), and the average of the marker score exponentially weighted ($1/f^2$). Both, SD_f and $1/f^2$ give high DI values to populations with relatively rare alleles. In the case of SD_f, the average of the scores of the most common allele among all populations was high (about 0.85) in both species. In $1/f^2$, the exponential weighting of allele scores makes the contribution of each locus to the DI increase by the square of its decrease in score. However, when DI was calculated by this alternate method (as the average of $1/f^2$), no significant associations with ecogeographical parameters were detected. Thus, in the interest of space, no details of results with respect to this statistic are presented. Rather, all results presented are with respect to DI calculated as SD_f.

Climatic information for each collecting site was obtained from public databases of the National Climatic Data Center-National Oceanic and Atmospheric Administration (NCDC-NOAA) (see Table 2). Collection records provided information on habitat features such as type of surrounding vegetation, population and plant size, latitude, longitude, altitude, physical distance, etc. (records are available through NRSP-6 homepage). Additionally, information on soil type, geological age of the sites, drought and freeze potential, and rodent concentration also was obtained (Cockrum, 1960; Davis and Schmidly, 1994; Fitzgerald et al., 1994). Since most of these records were included as discrete data (i.e., presence vs. absence, soil types, etc.), they were only used to determine associations with diversity coefficients.

To test for an association between genetic variation and ecogeographical variables, correlation and regression analysis were performed. PROC CORR and PROC REG subroutines of SAS-program were used (SAS Institute, 1992). Stepwise regression analysis also was performed to identify the variables

Table 1. List of *S. fendleri* and *S. jamesii* populations and their geographic locations used in this study. (More detailed information on these populations is available through the NRSP-6 homepage: http://www.ars-grin.gov/nr6.)

PI number	Collector's number	Latitude	Longitude	Altitude	Collection date	Number of Plants Collected
				m		
1) *S. fendleri*						
564024	SBV 01	32°25′	110°44′	2400	6 August 1992	16
564025	SBV 03	31°26′	110°19′	1900	7 August 1992	4
564026	SBV 04	31°56′	109°16′	2120	8 August 1992	18
564027	SBV 05	31°54′	109°16′	2480	8 August 1992	11
564028	SBV 06	31°53′	109°16′	2580	8 August 1992	13
564029	SBV 07	32°22′	106°33′	1850	9 August 1992	10
564030	SBV 08	32°57′	105°43′	2620	9 August 1992	12
564031	SBV 09	32°57′	105°43′	2620	9 August 1992	11
564032	SBV 10	32°56′	105°43′	2650	9 August 1992	15
564033	SBV 11	32°56′	105°43′	2680	9 August 1992	19
564034	SBV 12	32°59′	105°34′	2350	9 August 1992	9
564035	SBV 13	32°57′	105°35′	2540	10 August 1992	12
564036	SBV 14	32°57′	105°37′	2400	10 August 1992	9
564037	SBV 15	32°59′	105°42′	2580	10 August 1992	12
564038	SBV 16	33°28′	105°48′	2420	10 August 1992	8
564039	SBV 18	33°23′	108°46′	2100	11 August 1992	26
564040	SBV 19	33°24′	108°35′	2350	11 August 1992	7
564041	SBV 20	33°48′	108°28′	2500	12 August 1992	12
564042	SBV 23	33°47′	109°09′	2400	12 August 1992	11
564043	SBV 25	34°02′	109°09′	2350	12 August 1992	5
564044	SBV 30	34°13′	108°33′	2120	13 August 1992	1
564045	SBV 32	33°26′	105°43′	3200	4 August 1992	30
564046	SBV 33	32°01′	109°21′	1780	8 August 1992	15
578234	BAM 01	32°25′	110°44′	2320	5 October 1993	22
578235	BAM 02	33°23′	108°46′	2100	8 October 1993	15
585112	BAM 06	32°25′	110°44′	2200	24 September 1994	2
585113	BAM 07	32°01′	109°20′	1780	26 September 1994	2
585114	BAM 08	31°42′	110°52′	2700	24 September 1994	20
585115	BAM 09	32°42′	109°57′	2618	25 September 1994	16
592400	BAM 17	33°26′	105°43′	2938	25 September 1995	SS†
592401	BAM 18	33°24′	105°46′	3036	25 September 1995	1
592402	BAM 19	33°06′	105°38′	2100	25 September 1995	21
592403	BAM 20	32°57′	105°43′	2620	25 September 1995	6
592404	BAM 21	32°57′	105°43′	2620	25 September 1995	SS
592405	BAM 22	31°53′	109°16′	2580	26 September 1995	SS
592406	BAM 23	31°54′	109°16′	2480	26 September 1995	4
592409	BAM 26	32°54′	107°48′	2375	26 September 1995	2
592412	BAM 29	34°02′	109°09′	2350	27 September 1995	19
592415	BAM 32	33°47′	109°09′	2400	27 September 1995	2
592420	BAM 37	33°56′	108°39′	2140	27 September 1995	21
595774	BAM 41	32°54′	107°48′	2375	16 August 1996	18
595776	BAM 43	32°54′	107°45′	2508	16 August 1996	16
595779	BAM 46	30°41′	104°04′	1550	18 August 1996	13
595781	BAM 48	30°42′	104°06′	1550	18 August 1996	9
596520	HAMM 11067	34°01′	109°27′	2740	19 September 1996	15
B) *S. jamesii*						
564047	SBV 02	31°23′	110°21′	1850	7 August 1992	16
564048	SBV 17	32°29′	108°31′	1900	11 August 1992	12
564049	SBV 21	33°55′	108°28′	2150	12 August 1992	21
564050	SBV 22	33°41′	108°51′	1950	12 August 1992	1
564051	SBV 24	34°02′	109°09′	2350	12 August 1992	15
564052	SBV 26	33°59′	109°07′	2500	12 August 1992	13
564053	SBV 27	34°06′	109°14′	2240	12 August 1992	15
564054	SBV 28	34°08′	108°28′	2320	13 August 1992	5
564055	SBV 29	34°13′	108°33′	2120	13 August 1992	9
564056	SBV 31	34°06′	107°27′	2140	13 August 1992	11
564057	SBV 34	32°00′	109°22′	1780	8 August 1992	16
578236	BAM 03	33°41′	108°51′	1950	8 October 1993	5
578237	BAM 04	34°23′	110°34′	1830	9 October 1993	7
578238	BAM 05	34°26′	110°36′	1960	9 October 1993	6
585116	BAM 20	35°12′	107°44′	2225	27 September 1994	9
585117	BAM 11	35°12′	107°44′	2225	27 September 1994	1
585118	BAM 12	35°12′	107°44′	2225	27 September 1994	10
585119	BAM 13	37°09′	108°31′	1950	29 September 1994	10
592397	BAM 14	34°46′	106°14′	1980	24 September 1995	6
592398	BAM 15	34°17′	105°37′	1980	24 September 1995	6
592399	BAM 16	34°08′	105°43′	2070	24 September 1995	7
592407	BAM 24	32°00′	109°22′	1780	26 September 1995	2
592408	BAM 25	32°29′	108°31′	1900	26 September 1995	14
592410	BAM 27	32°54′	107°48′	2375	26 September 1995	28
592411	BAM 28	34°06′	109°14′	2240	27 September 1995	3
592413	BAM 30	34°02′	109°09′	2350	27 September 1995	3
592414	BAM 31	33°59′	109°07′	2500	27 September 1995	36

Continued on next page.

Table 1. Continued.

PI number	Collector's number	Latitude	Longitude	Altitude	Collection date	Number of Plants Collected
				m		
B) *S. jamesii*						
592416	BAM 33	33°43′	109°16′	2438	27 September 1995	15
592417	BAM 34	33°46′	108°42′	1890	27 September 1995	4
592418	BAM 35	33°56′	108°28′	2150	27 September 1995	16
592419	BAM 36	33°56′	108°39′	2140	27 September 1995	27
592421	BAM 38	34°13′	108°33′	2120	27 September 1995	6
592422	BAM 39	34°44′	107°58′	2100	27 September 1995	8
592423	BAM 40	34°56′	111°27′	2169	5 October 1995	21
595775	BAM 42	32°54′	107°45′	2508	16 August 1996	19
595777	BAM 44	32°25′	106°34′	1739	16 August 1996	6
595778	BAM 45	31°52′	106°29′	1800	16 August 1996	12
595780	BAM 47	30°42′	104°06′	1550	18 August 1996	19
595782	BAM 49	31°59′	104°50′	1900	19 August 1996	17
595783	BAM 50	35°05′	105°42′	2100	20 August 1996	11
595784	BAM 51	35°37′	105°41′	2098	20 August 1996	18
595785	BDM 52	37°08′	104°40′	1950	19 September 1996	24
595786	BDM 53	37°08′	104°40′	1950	19 September 1996	20
595787	BDM 54	37°42′	109°40′	1620	21 September 1996	34
595788	BDM 55	35°56′	107°06′	1950	22 September 1996	20
596519	WOR 25779	31°59′	108°12′	1700	7 September 1996	27
603051	BMP 56	37°46′	111°27′	1708	13 September 1997	29
603052	BMP 57	37°46′	111°37′	1708	13 September 1997	3
603053	BMP 58	37°59′	109°31′	2077	16 September 1997	4
603054	HOLM 01	37°02′	107°27′	1921	1 October 1997	4
603055	WHIT 01	36°01′	107°52′	1890	30 October 1997	32
603056	WHIT 02	36°01′	107°54′	1890	30 October 1997	15
603057	WHIT 03	36°03′	107°55′	1890	30 October 1997	5

† SS means that the original collection was made as seed, so there were thousands of original propagules.

that best predict genetic associations. For the regression analysis of GD, ecogeographical variables were divided in four groups (see Table 3) to have an adequate number of degrees of freedom (df) for error to test the model. This approach was taken because a multiple regression analysis with a large number of independent variables in the model can produce erroneous results. In such a case, very high but incorrect R-squares would be generated by virtue of an improper decrease in the number of df for error. To measure the strength of the relationship between variables, Pearson's correlation coefficient was used as a test of significance.

Table 2. Pearson's correlation coefficients between genetic distance and ecogeographical variables for *S. fendleri* and *S. jamesii* populations.

Ecogeographical variables	Correlation coefficients for *S. fendleri*†	Correlation coefficients for *S. jamesii*‡
latitude	0.06	−0.03
longitude	0.22	0.07
altitude	−0.09	−0.10
distance	0.21	0.01
avg var T (m)§	0.08	0.05
avg var T (y)¶	0.12	−0.07
avg max T (m)	0.17	0.02
avg max T (y)	0.19	0.04
avg min T (m)	0.10	0.07
avg min T (y)	0.13	−0.11
heat deg days (m)	0.23	0.02
heat deg days (y)	0.16	−0.08
cool deg days (m)	0.00	0.11
cool deg days (y)	0.01	0.00
rainfall 1 (m)	0.17	0.08
rainfall 1 (y)	0.17	0.21
rainfall 2 (m)	0.15	0.06
rainfall 2 (y)	0.17	0.12

† 903 comparisons.
‡ 1379 comparisons.
§ (m) monthly average.
¶ (y) annual average.
Abbreviations used: avg min T: average minimum temperature; avg max T: average maximum temperature; heat deg days: heating degree days; cool deg days: cooling degree days; rainfall 1: average rainfall database 1; rainfall 2: average rainfall database 2.

RESULTS

Analysis of RAPD Markers and Estimation of Genetic Distances

A total of 903 pairwise comparisons based on 109 polymorphic RAPD markers were made for *S. fendleri*. The average GD among all populations was 0.22 and the highest and lowest distances detected between two populations were GD = 0.60 and GD = 0.00.

For *S. jamesii*, a total of 1378 pairwise comparisons of 123 polymorphic RAPD markers indicated that among all populations the mean distance was GD = 0.21. The highest GD detected between two populations was GD = 0.40 and the lowest was GD = 0.01.

A phenetic tree based on the distance matrix gives a

Table 3. R-square and *P*-values calculated by multiple regression analysis of ecogeographical parameters and genetic distance as dependent variable.

	S. fendleri		*S. jamesii*	
Parameter	*R*-square	$P > F$	*R*-square	$P > F$
Geographical†	0.223	0.37	0.147	0.48
Rainfall‡	0.168	0.36	0.164	0.42
Cooling and heating degree days§	0.391	0.08	0.136	0.52
Temperature¶	0.324	0.40	0.263	0.38

† Latitude, longitude, altitude and physical separation.
‡ Average rainfall (monthly and annual).
§ Heating and cooling degree days (monthly and annual averages).
¶ Minimum, maximum and average temperature (monthly and annual averages).

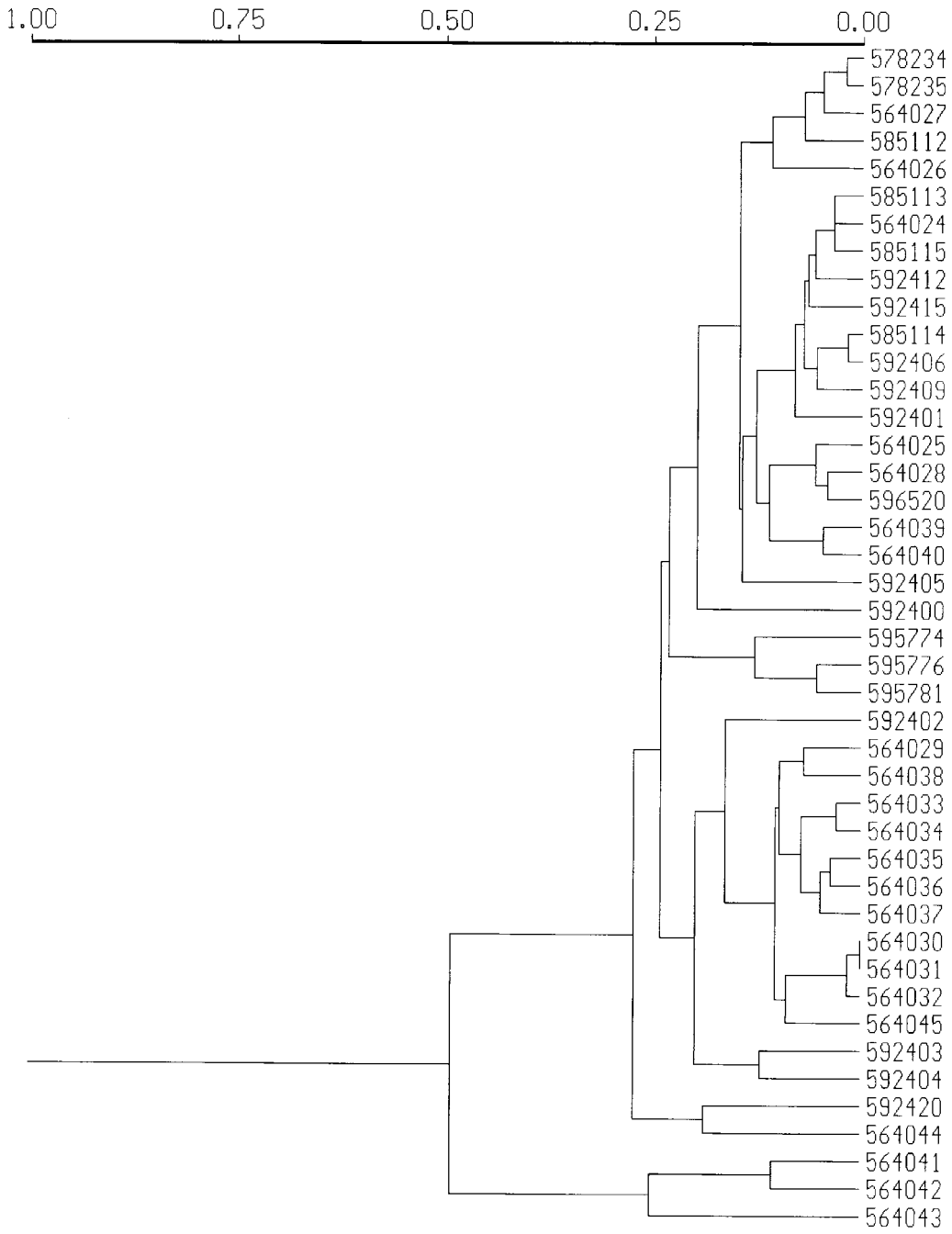

Fig. 1. UPGMA phenogram of genetic relationships among *S. fendleri* populations based on genetic distance coefficients determined by RAPD markers.

graphic representation of the relationship among populations for each species (Fig. 1 and Fig. 2).

Estimation of Genetic Diversity

Analysis of 109 RAPD loci among all 43 populations of *S. fendleri* showed that RAPD band frequencies among populations ranged from very rare 0.02 (1/43) to bands which were present in nearly all populations 0.98 (42/43). Mean genetic diversity among all populations was DI = 0.22. Genetic diversity ranged from a minimum DI = 0.10 in populations 585112 and 592412 to a maximum DI = 0.38 in population 564041.

RAPD band frequencies among 53 populations of *S. jamesii* varied from 0.11 (6/53) to 0.96 (51/53) for a total of 123 RAPD loci evaluated. Mean DI was 0.26, and genetic diversity ranged from DI = 0.21 in 592410 to DI = 0.32 in 592398.

Association between Ecogeographical Variables and Genetic Distance, GD

Correlation and regression analysis between genetic distance and ecogeographical variables for *S. fendleri* and *S. jamesii* populations showed that genetic distance was not significantly related to any of the ecogeographi-

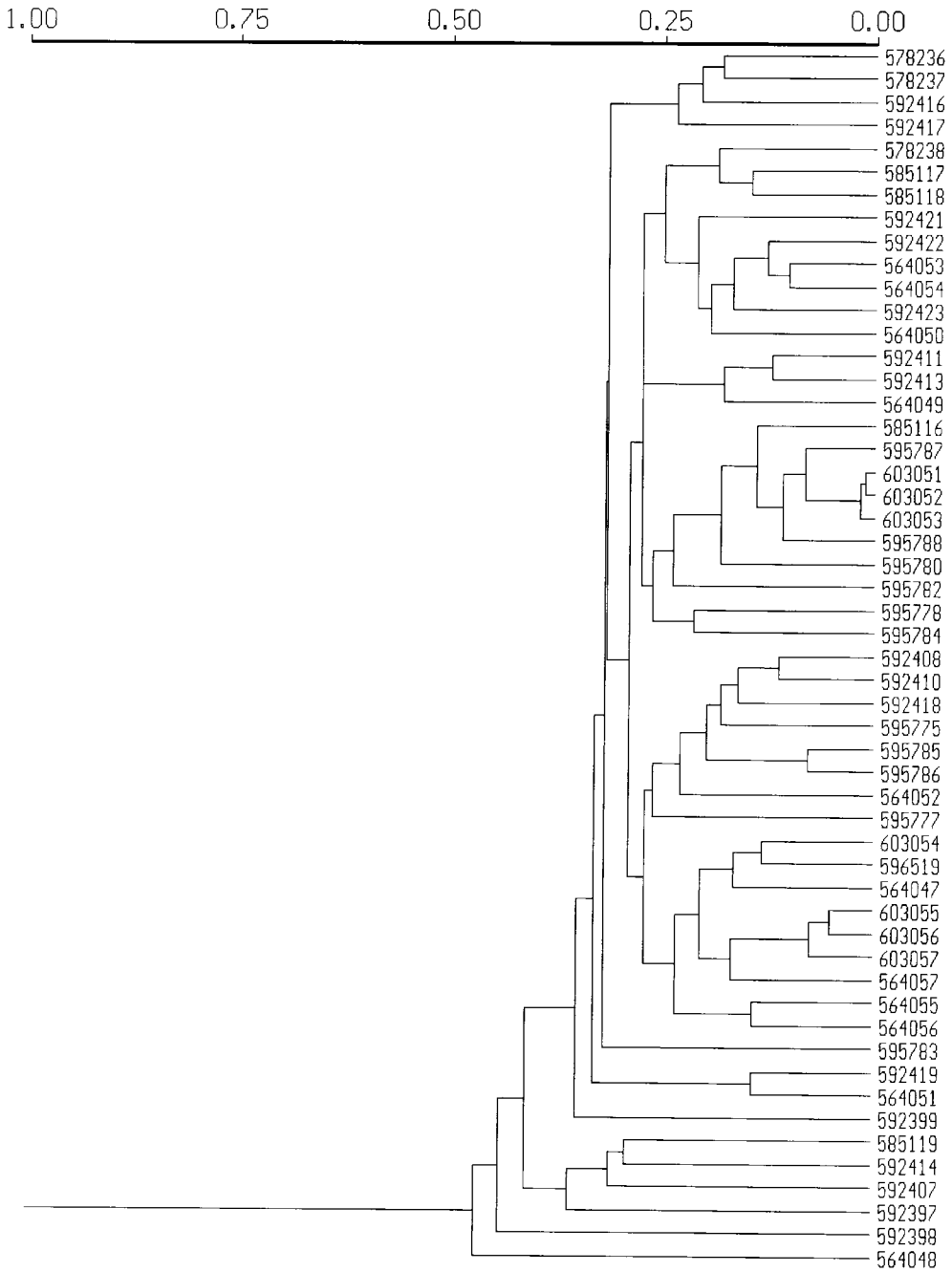

Fig. 2. UPGMA phenogram of genetic relationships among *S. jamesii* populations based on genetic distance coefficients determined by RAPD markers.

cal variables used in this study (Tables 2 and 3).

The multiple regression analysis including all variables revealed a maximum, but not significant $R^2 = 0.39$, $P = 0.08$ for cooling and heating degree day variables in *S. fendleri* populations. On the other hand, a maximum $R^2 = 0.26$, $P = 0.38$ was observed in *S. jamesii* for temperature variables (Table 3).

Association between Ecogeographical Variables and Genetic Diversity, DI

Solanum fendleri populations showed significant but low correlations between genetic diversity and latitude (0.39 $P = 0.00$), longitude (-0.41 $P = 0.00$), monthly average heating degree days (0.45 $P = 0.00$), the two sets of data of monthly and annual average rainfall (-0.32 $P = 0.04$, 0.34 $P = 0.02$, 0.33 $P = 0.03$, and 0.33 $P = 0.03$), and monthly and annual variation of temperature (0.42 $P = 0.03$ and 0.42 $P = 0.03$ respectively). The complete correlation analysis is presented in Table 4.

Multiple regression analysis in *S. fendleri* including all variables showed that genetic diversity was significantly predicted by the variables ($R^2 = 0.65$ $P = 0.04$). Stepwise regression analysis identified latitude, longitude,

Table 4. Pearson's correlation coefficients between genetic diversity† and ecogeographical variables for *S. fendleri* and *S. jamesii* populations.

Ecogeographical variables	*S. fendleri*	*S. jamesii*
Geographical		
latitude	0.39**	−0.15
longtitude	−0.41**	0.08
altitude	0.03	−0.15
Temperature		
average max T (m)	0.08	0.20
average max T (y)	0.12	0.18
average T (m)	−0.11	0.26
average T (y)	−0.13	0.19
average min T (m)	−0.23	0.24
average min T (y)	−0.25	0.13
variation T (m)	0.42**	0.15
variation T (y)	0.42**	0.09
Degree days		
heating degree D (m)	0.45**	0.07
heating degree D (y)	−0.05	0.20
cooling degree D (m)	−0.31	0.22
cooling degree D (y)	−0.22	0.17
Rainfall		
average rainfall 1 (m)	−0.32*	−0.09
average rainfall 1 (y)	0.34*	−0.03
average rainfall 2 (m)	0.33*	−0.25
average rainfall 2 (y)	0.33*	−0.06
Soil age		
cretaceous	0.05	−0.03
lower tertiary	−0.23	−0.16
quaternary & tertiary	−0.15	−0.14
upper tertiary	−0.19	−0.10
jurassic & triassic	0.05	0.04
upper paleozoic	0.27	0.02
Type of soil		
alluvial soil	N/A	−0.02
argid	−0.34	0.11
black soil	−0.23	0.21
culvert/ditch	N/A	−0.17
dark humus	−0.02	0.15
entisol	N/A	−0.23
grass	0.08	−0.33
gravel	0.27	0.24
leaf mulch	−0.16	0.02
orthids	0.05	−0.00
sandy dry	−0.02	0.13
sandy moist	0.11	0.06
ustolls	0.34	0.02
volcanic soil	−0.04	0.05
Potential to freeze		
freeze free	N/A	−0.21
Drought potential		
dry-arid	N/A	0.06
dry-marginal	N/A	−0.02
Surroundings		
rocks	0.14	−0.14
ivy	N/A	−0.16
creek bank	−0.18	−0.03
junipers	−0.20	0.01
cottonwood	N/A	−0.14
ponderosa	−0.18	−0.03
chenopodium	N/A	−0.05
cupress	N/A	0.10
rotten logs	−0.14	N/A
cattle manure	−0.06	N/A
Plant size		
plant size small	0.03	−0.22
plant size medium	0.00	−0.07
plant size large	0.11	0.17
Population size		
large	−0.09	0.24
small	0.22	−0.18
medium	−0.16	−0.12
Rodent density		
high	N/A	−0.21
medium	−0.05	−0.03
low	0.05	0.15

* indicates significance at $P = 0.05$.
** indicates significance at $P = 0.01$.
N/A = No population collected in these areas.
† Based on DI calculated as SD_f.

monthly average heating degree days, and monthly variation of temperature as the most important predictors of genetic variation ($R^2 = 0.39$ $P = 0.00$).

In *S. jamesii*, no significant correlations were observed between genetic diversity and ecogeographical variables. Multiple regression analysis, including all variables showed no significant association ($R^2 = 0.26$, $P = 0.76$), stepwise analysis determined annual average temperature and annual average maximum temperature were most important predictors; however, these were very weak ($R^2 = 0.09$, $P = 0.10$).

DISCUSSION

Physical Proximity Not Associated with Genetic Relationships

RAPD markers determined that genetic differences exist among populations of *S. jamesii* and *S. fendleri* (Fig. 1 and Fig. 2). However, these patterns of genetic differentiation were not well associated with the ecogeographical variables assessed. Remarkably, even physical proximity of populations was not a very good predictor of genetic resemblance. This particular finding could be of importance in planning future explorations for collecting potato species. Marshall and Brown (1975) pointed out that guidelines for collecting emphasize the use of geographical separation for determining sampling sites. Chapman (1989) indicated that collection sites must be as diverse, geographically and ecologically, as possible. Under this scheme, populations closely located within an area may not be considered worth collecting because redundant genotypes are expected to be found. Some previous research in other species has found that genetic similarity is very closely associated with geographical proximity (Francisco-Ortega et al., 1993; Hormaza et al., 1994), while others failed to detect a good relationship (Fahima et al., 1999; Gallois et al., 1998). Loveless and Hamrick (1984) explained that populations in close neighborhoods tend to be uniform because genetic differentiation is often prevented by gene flow. Though some potato populations in close proximity were found to be highly related, i.e., *S. fendleri* 564030-564031 GD = 0.00, 564032-564033 GD = 0.05 and, *S. jamesii* 603051-603052 GD = 0.01, some were not, i.e., *S. fendleri* 592415-564042 GD = 0.31; 592412-564043 GD = 0.23, 592400-564045 GD = 0.23; *S. jamesii* 564048-592408 GD = 0.27, 564051-592413 GD = 0.23, 564053-592411 GD = 0.21, 564057-592407 GD = 0.21. Fahima et al. (1999) studying genetic variation in wild emmer wheat [*Triticum dicoccoides* (Koern. ex Asch. &Graebner) Aarons.] populations also found similar results. Moreover, they pointed out that quite often it was easier to find a greater genetic difference between close populations than among populations which are far apart.

Hamrick (1987) determined that characteristics of habitats may be quite heterogeneous within small areas. That concept can explain why genetic differentiation among populations is independent of distances. Cobb et al. (1994), Mopper et al. (1991), and Mitton et al. (1998) reported consistent patterns of microgeographic variation among pinyon populations at Sunset Crater,

Arizona. Mitton et al. (1998), studying allozyme variation in pinyon (*Pinus edulis* Engelm.) populations, observed a repeated pattern of microgeographic variation between moist and dry sites. For instance, allele three of glycerate dehydrogenase (*Gly-3*) showed a higher frequency on dry sites. Fahima et al. (1999) reported that a sharp local differentiation, rather than a gradual change, in allele frequencies across the geographical range explains the lack of association between genetic distance and separation. The absence of a significant relationship between geographical separation and genetic distance might also indicate that microgeographic (local) variation is a significant factor of genetic differentiation (Antonovics and Bradshaw, 1970; Antonovics, 1971). Most often, potato populations in the USA, like those in Latin America (D.M. Spooner, 1999,personal communication), are very small (<100 plants) and isolated. For this reason, the actual microclimate differences relevant to populations may not be well represented by the data based on broader areas used here. Under such conditions, stochastic events may have extreme effect in modifying genetic structure of populations. Events such as environmental changes, demographic factors (i.e., chance differences among individuals in survivorship or fecundity), and genetic drift are likely to have greater repercussions.

Limits of Resolution Due to Sampling Errors

Genetic differences also may be explained by sampling error. *Solanum jamesii* is a diploid outcrosser with self-incompatibility. Populations having this type of breeding system are expected to produce highly heterozygous and heterogeneous populations (Loveless and Hamrick, 1984). Thus, many individuals (genotypes) should be pooled from each population to assure that a representative sample has been taken (del Rio and Bamberg, 1998). Previous results of the authors (del Rio et al., 1997b) however, show that this is not observed in wild *S. jamesii* since plants within samples of these populations are very homogeneous. It is possible that samples taken in different seasons tend to be different homogeneous subsets of an overall heterogeneous population, their genetic identities being determined by the particular time and conditions at collection. This is supported by the observation that ''duplicate'' samples (i.e., from exactly the same place but collected in different seasons) were not genetically identical (GD equals about 0.17 for both species).

Thus, imprecision in both the genetic and ecogeographical characterization associated with particular collection sites could have obscured real significant correlations between the two. This analysis, however, seeks practical conclusions, and such imprecision is currently a practical reality.

Significant Associations with Genetic Diversity

Although parameters of ecogeographical structure measured here did not explain genetic differentiation, a significant association of genetic diversity with ecogeographical variables was detected in *S. fendleri* populations when DI was calculated as SD_f.

For *S. fendleri*, associations with latitude and longitude suggest that populations from northeastern regions of the range tend to be more diverse than populations from other regions. This pattern of diversity was also associated with some climatic variables, suggesting that these regions have distinctive environments which promote the emergence of rare alleles.

When DI was calculated as the average of exponentially weighted marker scores ($1/f^2$), no significant correlations with ecogeographical parameters were detected. This also held true when a variety of exponents other than 2 were used (data not presented). Although no method of calculating DI resulted in strong correlations with any ecogeographical parameter, the DI statistic still has practical use. A high DI identifies populations that merit special attention in the genebank. Their relative abundance of rare alleles means that they make a greater contribution to the overall genetic diversity of the collection than most populations do. Also, by studying the sites of origin of populations with high DI values, one might discover a previously unrecognized ecogeographical parameter the populations have in common, and thereby identify other sites with the same parameter. Such new sites could be the richest sources for collecting new diversity.

Immigration also may explain differences in diversity. Slatkin (1987) indicated that selection tends to adapt a population to local environmental conditions but that immigrants also contribute genes adapted to other conditions. Human intervention can account for immigration. Moeller and Schaal (1999) indicated that trade among Native Americans might have been responsible for the great morphological variation among maize accessions in the Great Plains. Wild potatoes were consumed by Native Americans and movement of tubers along the trade routes of the Anasazi may have occurred. Populations of *S. fendleri* might be in a transition process in adaptation to new environments, which would explain why they are rich in rare alleles.

These populations are assumed to have moved progressively northward by natural and artificial means. Migration to new locations mediated by birds or other animals could also foster differentiation. These locations may be rare oases where conditions favorable to potato survival exist. Thus, many distinct colonies could have been brought from far away, maintaining their genetic differences *clonally*. For example, birds may have come from diverse sites and converged in one area (i.e., attracted by water or food), "planting" several potato genotypes.

In summary, assessment of interpopulation genetic distances in both species showed no associations with ecogeographical variables, suggesting that genetic differentiation is predicted by factors more subtle than those assessed here. However, significant associations between diversity and ecogeographical variables in *S. fendleri* indicate that collecting across a specific range of geographical coordinates may result in maximizing the acquisition of representative samples of the gene pool for this species.

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